

Membrane-Active Peptides and Toxins II

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Novel Lipid Dynamics Around the Cytolysin-A Membrane-Pore Complex and its Intermediates

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Pore forming toxins (PFTs) are ubiquitous weapons in the armoury of many organisms. It is often observed that small numbers of these potent proteins form stable pores, permeabilize the cell membrane and cause cell lysis. The complex role of the membrane in the formation of these pores, the influence of the pore on the structure and dynamics of the surrounding membrane and the expulsion of the central lipids upon pre-pore formation are important phenomena that are poorly understood due to the paucity of structural data.

Multi-scale molecular dynamics was carried out on the PFT Cytolysin-A (ClyA), to address its interaction with the surrounding lipid membrane. A large heterogeneity in the lipid self-diffusivities were observed with the presence of more mobile and less mobile lipid fractions spanning the membrane. These results indicate that the local environment around the protein complex is markedly different from the rest of the membrane. Simulations of intermediate ClyA oligomers in a variety of membranes shows rapid evacuation of the central lipids from the interior to the free membrane surrounding the partially formed pore assembly. This implies that concerted lipid expulsion occurs prior to the formation of the dodecameric pore complex. This alternative hypothesis challenges the notion that destabilization and ejection of a membrane patch corresponding to the pore-lumen occurs after the pre-pore assembly on the membrane surface is complete. This mechanism could occur across PFT families and has implications on membrane resealing and cell recovery.

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How Many AMP Molecules Kill a Bacterium? Spectroscopic Determination of PMAP-23 Binding to *E. coli*

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Antimicrobial peptides (AMPs) kill bacteria mainly through the permeabilization of the plasma membrane. Experiments on these molecules generally focus either on their biophysical characterization in model membranes, or on their activity on bacterial cells, but studies demonstrating a correlation between biological activity and behaviour in liposomes are still lacking.

One unanswered issue is the minimal amount of bound peptide that is necessary to kill a bacterial cell. Different attempts to assess this quantity [1, 2] reached different conclusions, probably because the fraction of peptide bound to bacteria is usually extrapolated based on binding experiments performed on liposomes.

Trying to fill the hiatus between biological and biophysical studies, we determined by fluorescence measurements the affinity of a dansyl-labeled analogue of the PMAP-23 AMP [3] for both liposomes and *E. coli* cells. Experiments were performed in the peptide concentration range that displays bactericidal activity.

These results will provide a direct determination of the minimal number of peptide molecules which are necessary to kill a bacterial cell.

[1] ACS Chem. Biol. 2010, 10: 905.

[2] Nat. Rev. Microbiol. 2009, 7: 245.

[3] Biochim. Biophys. Acta 2009, 1788: 1523.

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Length-Dependent Activity of Membrane-Bound Cationic Amphipathic Alpha-Helical Peptides

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MSI-103 [sequence (KIAGKIA)₃-NH₂] is a designer-made antimicrobial peptide based on the sequence of PGLa, a host defense peptide from the African frog *Xenopus laevis*, with high activity against bacteria [1, 2]. It forms an amphipathic α -helix upon binding to a lipid bilayer, and has been proposed to kill bacteria by forming membrane pores. If this were the case, shorter analogs that are not long enough to span the membrane should not be able to form pores and should be inactive. To test this hypothesis we have synthesized a

series of analogs of MSI-103, called KIA peptides, with a length of 14 to 28 amino acids, all of which were shown to be α -helical by circular dichroism spectroscopy. We tested their antimicrobial and hemolytic activities and the ability to induce vesicle leakage, and found that there is a threshold length needed for activity, supporting the pore formation hypothesis. Using solid-state ¹⁵N-NMR on isotopically labeled peptides, we also investigated the orientation of the different KIA peptides in membranes of different lipid composition, and also here observed a systematic dependence on peptide length.

References:

[1] J. Blazyk, R. Wiegand, J. Klein, J. Hammer, R.M. Eppard, R.F. Eppard, W.L. Maloy, U.P. Kari, J. Biol. Chem., 276 (2001) 27899-27906.

[2] E. Strandberg, N. Kanithasen, J. Bürck, P. Wadhvani, D. Tiltak, O. Zwer-nemann, A.S. Ulrich, Biochemistry, 47 (2008) 2601-2616.

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Solid-State NMR Structure Analysis of the Short Multifunctional Peptide BP100 in Membranes

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The 11-residue peptide BP100 (KKLFKKILKYL-amide) is a short antimicrobial peptide that has been optimized against plant pathogens, and it is also able to act as a cell-penetrating agent. To address the conformation, orientation and dynamics of BP100 in the membrane-bound state, we have labeled all of the six hydrophobic amino acids individually with CF₃-L-Bpg for solid-state ¹⁹F-NMR analysis, and a ¹⁵N-label was incorporated at Leu8. Circular dichroism (CD) analysis showed that the substitutions did not perturb the overall structure of the α -helical peptide, and the antimicrobial activity also remained unaffected. Using highly sensitive ¹⁹F-NMR, we found that the orientation of BP100 in macroscopically oriented DMPC/PG membranes remains unchanged as a function of peptide concentration over a wide range of peptide-to-lipid molar ratios from 1:10 to 1:3000, accessible only by fluorine NMR. The ¹⁹F-NMR data analysis was compatible with a large family of possible helix tilt angles, but these could be narrowed down by including peptide dynamics in the structure determination process. These results were complemented by ¹⁵N-NMR and oriented CD spectroscopy, both showing that the amphiphilic BP100 helix is oriented parallel to the membrane surface. In summary, our results show that the short BP100 molecule assumes a surface bound state under different concentration regimes and remains highly mobile. We suggest that at low peptide concentration BP100 may be able to permeate cellular membranes in a transient way, but at high concentration it perturbs the membrane via a carpet mechanism.

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How SpoVM Interacts with Lipid Bilayers and Bacterial Cell Membranes

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SpoVM, a small protein with 26 residues, is essential for spore formation in *Bacillus subtilis*. SpoVM is produced in the mother cell chamber of the sporangium during the process of sporulation and is recruited to the polar septum after the sporangium undergoes asymmetric division. There are reports suggesting that SpoVM localizes to the surface of the forespore by sensing membrane curvature. Besides its transformation from random coils to α -helices when binding to a lipid bilayer, the molecular mechanism of how SpoVM interacts with membranes has not been clarified. For example, we found that the curvature dependence of the binding affinity is difficult to detect. By using single giant unilamellar vesicle method, we found that SpoVM binds to the membrane and expands the surface area of membrane at low concentration with no molecular leakage from the vesicle. However, above the critical concentration, SpoVM causes leakage of the content dye from the GUV. The critical concentrations for different lipid compositions including phosphoethanolamine, phosphatidylglycerol, phosphatidylcholine and cardiolipin were further investigated. Surprisingly, the critical concentration for GUVs with 30% PE is more than six times higher than for other lipid compositions without PE. To understand how SpoVM interact with bacterial membranes, fluorescent microscopy was used to directly observe the effect of SpoVM on *Escherichia coli* in real time. The result showed that SpoVM makes both the outer membrane and the cytoplasmic membrane of *Escherichia coli* permeable to Sytox Green above a critical concentration. Oriented circular dichroism results show that SpoVM changes the orientation of its helical axis from parallel to perpendicular with respect to the plane of bilayers. Our results suggest that the SpoVM-membrane interaction follows the same pattern as many other helical antimicrobial peptides, such as magainin, melittin and islet amyloid polypeptides.